

ANALYTICAL METHODOLOGY FOR DETERMINATION OF DIPHENYLAMINE ANTIOXIDANTS IN WASTEWATER / BIOSOLIDS AND SEDIMENT

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Introduction

Diphenylamine derivatives are widely used as antioxidant additives in vehicle engine oils, commercial/ industrial lubricants and in products composed of rubber. These substances were identified as a high priority for screening assessment and were included in the challenge because they were found to meet the ecological categorization criteria for persistence, bioaccumulation potential and inherent toxicity to aquatic organisms^{1,2}. Benzenamine, N-phenyl-, reaction products with styrene and 2,4,4-trimethylpentene (BNST, 68921-45-9) produced from these reactions are mixtures of diphenylamines with each mixture differing by the type and extent of substitution. These substances meet the ecological categorization criteria for persistence, bioaccumulation potential and inherent toxicity to non-human organisms.

Releases of BNST to soil, municipal wastewater and surface water are expected due to industrial wastewater discharge, leaks and spills from lubricants as well as from the improper disposal of used engine oils and lubricants. At this point, there are few existing environmental measurements of these substances in any media³. In this study, we developed a method for the determination of eight BNST (two styrenated-diphenylamine (S-DPA) isomers, isooctyl-diphenylamine (TO-DPA), three isooctyl-styrenated-diphenylamine (TOS-DPA) isomers, diisooctyl-diphenylamine (DTO-DPA), diisooctyl-styrenated-diphenylamine (DTOS-DPA)) mixtures in mediums such as wastewater, biosolid and sediment using gas chromatography-tandem mass spectrometry (GC-MS/MS). The method was applied in the analysis of sediment, wastewater and biosolids collected from various southern regions of Canada. To the extent of our knowledge, this is the first work to report the determination of diphenylamine antioxidants in environmental samples.

Materials and methods

Chemicals and Reagents. ¹³C₁₂-Diphenylamine (with a purity of 99%) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Alkylated diphenylamines (8 BNST components; CAS#: 68921-45-9; technical grade) were purchased from AK Scientific (Union City, CA, USA).

Sample Collection. Influent and effluent wastewater samples were collected in July, 2013 from 8 Wastewater Treatment Plants (WWTPs) in Canada. Influent and effluent wastewater samples were collected as 24-hour equal volume composites (200 mL every 15 min) using a refrigerated auto sampler (HACH Company; Loveland, Colorado, USA). Biosolid samples were centrifuged and moisture content was less than 85% for wet samples.

Sample Preparation. One liter of effluent sample or 0.3 L influent sample was spiked with the surrogate, ¹³C₁₂-DPA and consecutively extracted three times (100+50+50 mL) with dichloromethane. The combined extracts were dried over anhydrous Na₂SO₄, solvent exchanged to iso-octane, and concentrated to 1~2 mL. A column containing 10 g of 5% deactivated silica gel topped with 2 g of anhydrous Na₂SO₄ was prepared and pre-rinsed with 25 mL of hexane. The sample extract was then applied onto the silica gel column and the first fraction was eluted with 25 mL of hexane. This fraction was discarded and the second fraction was eluted with 45 mL of a 1:1 mixture of ethyl acetate/hexane. The second fraction was concentrated and solvent exchanged with isooctane. It was then reduced under nitrogen to 1 mL for GC-MS/MS analysis. A 0.3~0.5 g biosolid (centrifuged, ~80%moisture content) sample was ground with 12~15 g of Na₂SO₄ and transferred into a 50 mL glass tube. The surrogate, ¹³C₁₂-DPA was then added and after 30 min of equilibration, 25 mL of a solvent mixture composed of hexane/acetone (1:1) was added. The extract was vortexed for 30 min, centrifuged at 2500 rpm for 5 min and the supernatant transferred into a flask. The extraction was repeated two more times and extracts

were pooled. Five mL of isooctane was added to the extract and concentrated by rotary evaporation and nitrogen to approximately 1.5 mL prior to a column cleanup.

Instrumental Analysis. An Agilent 7890A GC system (equipped with an Agilent 7693 auto sampler) interfaced with an Agilent 7000A Triple Quad Mass Spectrometer (GC-EI-MS/MS) was used for measurement of diphenylamine derivatives. Chromatographic separation was accomplished by use of a fused silica DB-5 MS capillary column (30 m, 0.25 mm, i.d., 0.25 μ m film thickness). The scan type was Multiple Reaction Monitoring (MRM); the MRM transitions monitored are given in **Table 1**.

Identification of the main components in BNST was accomplished by direct infusion atmospheric pressure photoionization (APPI⁺)-Frontier Transform Ion Cyclotron Resonance Mass Spectrometer (FT-ICR-MS) and GC-MS/MS. An Agilent 7890A GC system interfaced with a flame ionization detector (GC-FID) was used to calculate the relative area percentage of each peak in technical mixtures.

Results and discussion

Identification and Quantification. GC-MS was used for full scan of 100 ppm BNST (technical grade) standard and 9 major peaks were observed. The molecular ions (m/z) were 169.0, 273.1, 281.1, 385.2, 393.2 and 497.3, respectively. There were 2 peaks (S-DPA1 and S-DPA2) with mass 273.1 at retention times (RT) of 12.674 and 15.556 min. The molecular formula (MF) was $C_{20}H_{19}N$, indicating one C_8H_9 substitution on the diphenylamine rings (p- or o-). Three peaks (TOS-DPA 1, TOS-DPA 2 and TOS-DPA 3) with a mass of 385.2 were found at RT of 18.348, 19.437 and 22.337 min. The MF of $C_{28}H_{35}N$ indicates one C_8H_9 and one C_8H_{17} substitution on the diphenylamine rings (p-,p-; p-,o-; o-,o-). In BNST (**Fig. 1**), compounds which have the exact mass (EM) of 377.2134 and 489.3379 were distyrenated-DPA (two C_8H_9 substitution on the diphenylamine rings) and distyrenated-tertoctyl-DPA (two C_8H_9 substitutions and one C_8H_{17} substitution on the diphenylamine rings), their MFs were $C_{28}H_{27}N$ and $C_{36}H_{43}N$.

BNST standards was quantified using the relative area percentage of the peaks determined by GC-FID and the relative abundance of ions measured using FT-ICR-MS analysis.

Analysis of Wastewater and Biosolids. Each component in BNST spiked at 100- and 1000-ng (total concentrations) levels in effluent wastewater, influent wastewater and biosolids passed through the entire analytical procedure ($n=6$) with recoveries ranging from 70.5% to 112%, with a relative standard deviation (RSD) of 1.62 to 10.6%. The recoveries of surrogate ($^{13}C_{12}$ -DPA) spiked in sediment, effluent, influent and biosolids samples were 83.4% to 107% (mean: 93.3, RSD: 7.41%).

Few studies have reported the occurrence of diphenylamine antioxidants in wastewater, biosolids and sediment samples. In our study, we determined concentrations of 9 BNST components using GC-MS/MS in 16 wastewater samples (8 influent and 8 effluent), 5 biosolids from different WWTPs in Canada. All the target compounds were detected in all influent, effluent and biosolids samples collected from southern Canada ranging from 61.9~658 ng L⁻¹, 1.06~20.6 ng L⁻¹ and 218~958 ng g⁻¹ dw. Almost all the diphenylamine derivatives were detected in influent wastewater excluding TOS-DPA 2 and DTOS-DPA, and the derivatives were detected in biosolids samples. Dominant component of BNST both in influent and biosolids was DTO-DPA and their median concentrations were 35.9 ng L⁻¹ and 207 ng g⁻¹ dw, respectively.

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References

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Table 1. Elemental Composition and Transitions Monitored for Diphenylamine Derivatives Analyzed by GC-MS/MS.

Compounds	CAS No.	Elemental composition	Molecular weight	Retention time (min)	Transitions (m/z)	Collision energy (eV)
¹³ C ₁₂ DPA	-	¹³ C ₁₂ H ₁₁ N	181	7.712	181.1→180.3, 179.3	30
DPA	122-39-4	C ₁₂ H ₁₁ N	169	7.712	169.1→168.1, 167.1	20
S-DPA1	-	C ₂₀ H ₂₀ N	273	12.674	273.1→180.1, 258.2	20
TO-DPA	-	C ₂₀ H ₂₇ N	281	12.777	210.1→195.1, 180.1	20
S-DPA 2	-	C ₂₀ H ₂₀ N	273	15.556	273.1→258.2, 166.1	25
TOS-DPA 1	-	C ₂₈ H ₃₅ N	385	18.348	314.1→105.1, 206.1	25
TOS-DPA 2	-	C ₂₈ H ₃₅ N	385	19.437	314.1→206.1, 105.1	35
DTO-DPA	-	C ₂₈ H ₄₃ N	393	19.799	322.2→251.2, 105.1	20
TOS-DPA 3	-	C ₂₈ H ₃₅ N	385	22.337	314.1→105.1, 284.2	25
DTOS-DPA	-	C ₃₆ H ₅₁ N	497	24.288	426.2→355.2, 340.2	20

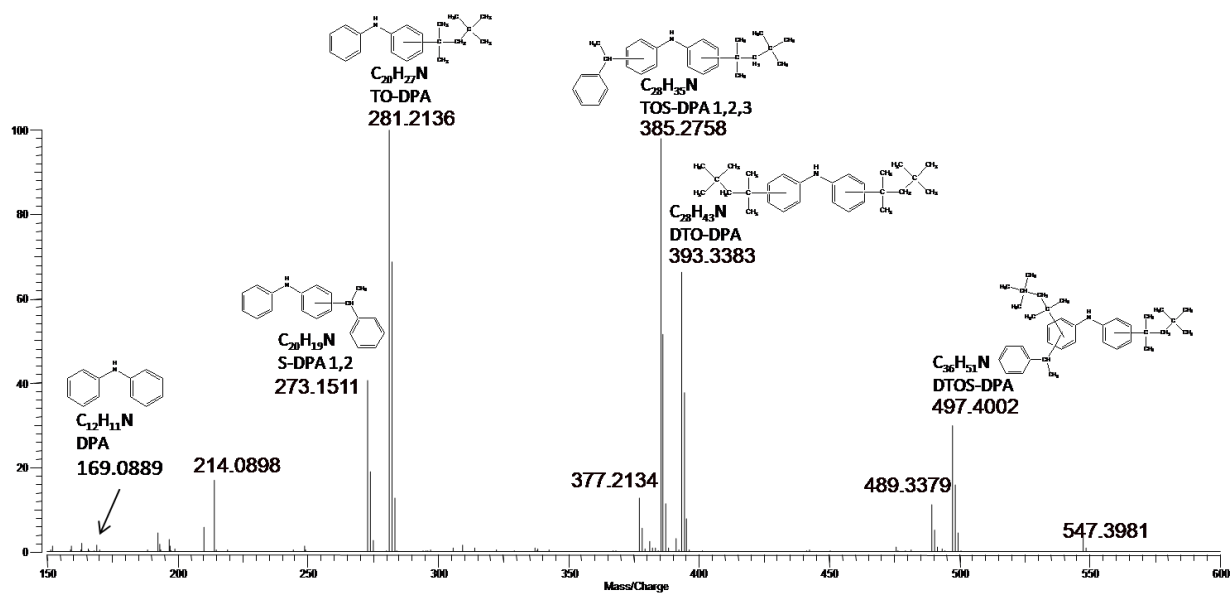


Fig 1. Mass spectrum of FT-ICR-MS